while Tumour Necrosis-α-like Weak inducer of Apoptosis (TWEAK) and interferon-β were increased over17d. No changes occurred in expression of mRNA for RANK, NFATc1, OSCAR or cathepsin-K.
Conclusions: The results show that bone resorption is inhibited by OST and its component RNAs in a concentrated-related manner. The inhibitory effects may possibly relate to events downstream of NFATc1 regulation.

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THE CINOD NCX 429 EXERTS ANTI-INFLAMMATORY EFFECTS IN ISOLATED HUMAN MONOCYTES AND MACROPHAGES

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Purpose: Cyclooxygenase-inhibiting nitric oxide donators (CINODs) represent a new class of anti-inflammatory drugs designed to provide the anti-inflammatory effects of non steroidal anti-inflammatory drugs (NSAIDs) while donating nitric oxide (NO) with the aim of mitigating side-effects in the gastrointestinal and cardiovascular systems. CINODs exercise their analgesic and anti-inflammatory effects via cyclooxygenase (COX) inhibition, and exhibit an improved tolerability, via the protective action of nitric oxide (NO) on gastric mucosa and vascular function. We have assessed the effect of NCX 429, a naproxen-based CINOD, on human monocytes and monocyte-derived macrophages (MDM) isolated from healthy volunteers, in comparison with naproxen.
Methods: Human monocytes were isolated from heparinised venous blood of healthy donors by standard techniques in Hystopaque gradient centrifugation (400×*g*, 30 min, room temperature) and recovered by thin suction at the interface. MDM were prepared from monocytes, by culture (8-10 days) in RPMI 1640 medium containing 20% FBS.
Cells (1×10^6) were pre-treated for 1 h with NCX 429 or naproxen (1 nM - 100 μM) and then stimulated with 0.1 μM phorbol myristate acetate (PMA) for 24 h. Supernatant was collected at the end of the incubation for measurement of IL-6 release (ELISA).
To evaluate superoxide anion (O_2^-) production, human monocytes (1×10^6 cells/plate) were stimulated with PMA (1 μM, 30 min) in the absence or presence of NCX 429 or naproxen. O_2^- production was evaluated by the superoxide dismutase (SOD)-inhibitable cytochrome C reduction and expressed as nmol cytochrome C reduced/10^6 cells/30 min. The MMP-9 activity was evaluated by gelatin zymography.
Results: NCX 429 in the range 1 nM - 100 μM inhibited PMA-induced IL-6 release in monocytes in a concentration dependent mode (IC_{50} = 870 nM); at the maximal concentration, the inhibition by NCX 429 reached 62±8%, significantly higher than the inhibition afforded by naproxen (36±4%, $p<0.05$, n=7). In MDM, 100 μM NCX 429 inhibited by 52±11% (n=5) PMA-induced IL-6 release, while naproxen had no effect. A stronger effect of NCX 429 was also observed by evaluating superoxide production in monocytes and MDM. At 1 μM, NCX 429 inhibited PMA-induced O_2^- production by about 24% in monocytes and 60% in MDM (n =6), whereas naproxen produced little or no effect. Moreover, NCX 429 reduced PMA-induced O_2^- production in monocytes isolated from patients affected by rheumatoid arthritis (n=3). Interestingly, NCX 429, but not naproxen, significantly inhibited matrix metalloproteinase (MMP)-9 activity in lipopolysaccharide (LPS)-challenged monocytes from healthy volunteers ($p<0.05$ *vs* LPS, n=3).
Conclusions: These results show additional anti-inflammatory effects of NCX 429 with respect to the reference NSAID, naproxen. CINODs, through NO donation, have been shown to have a favourable blood pressure and gastrointestinal profile relative to available NSAIDs in animal models; the present data suggest that NO donation from CINODs might also account for further anti-inflammatory effects beside the ones dependent on COX inhibition.

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NAPROXCINOD, UNLIKE NAPROXEN, INHIBITS EXPRESSION OF iNOS IN ACTIVATED MACROPHAGES IN VITRO

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Purpose: Macrophages play a pivotal role in several inflammatory diseases and represent a valuable model for studying anti-inflammatory properties of new drugs. Activated macrophages produce and release numerous inflammatory mediators including cytokines, prostaglandins and reactive oxygen and nitrogen species. At physiologic concentrations, nitric oxide (NO) inhibits the expression and activity of pro-inflammatory mediators, whereas at high concentrations (i.e., during inflammation) it induces cytokine expression and tissue damage. Naproxcinod is the first-in-class cyclooxygenase inhibiting nitric oxide donator (CINOD), designed to exert effective anti-inflammatory actions through cyclooxygenase (COX) inhibition while releasing NO, with the aim of mitigating NSAIDs-associated unwanted effects. The purpose of this study was to characterize COX-inhibiting and NO-donating properties of the CINODs naproxcinod and NCX 429, and to investigate whether they modulate the expression of inducible nitric oxide synthase (iNOS) in activated macrophages differently from a reference NSAID, naproxen.
Methods: NO release was evaluated by assessing vascular tone on isolated rabbit aortic rings pre-contracted with methoxamine (3 μM) was recorded after incubation with vehicle or test compounds (0.01-100 μM) in the presence or absence of the soluble guanylyl cyclase (sCG) inhibitor ODQ (1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one, 10 μM).
To assess COX-1 inhibition, RAW 264.7 murine macrophages were incubated with test compounds (0.01-100 μM, 30 minutes) and stimulated with arachidonic acid (1 μM, 15 min). To assess COX-2 inhibition, aspirin (100 μM) pretreated cells were incubated with 1 μg/ml LPS and 10 ng/ml IFNγ.for 16 h to induce COX-2 expression and then treated as above. Esterases (1.67 U/sample) were added to accelerate naproxcinod metabolism. Supernatant was collected for enzymatic immuno-assay of PGE_2.
In different experiments, cells were incubated overnight with LPS (1 μg/ml) and IFNγ (10ng/ml) in the presence of vehicle or test compounds (0.1-100 μM) and nitrites quantified in the supernatant by the Griess reaction. Cells were lysed for Western blot and iNOS quantified by chemiluminescence.
Results: Naproxcinod and naproxen showed similar *in vitro* inhibition of COX-1 and COX-2 (COX-1 IC_{50}= 3.0±1.3 and 8.1±5.8 μM, respectively; COX-2 IC_{50}= 3.9±1.7 and 10.4±8.4 μM, respectively). Additionally, naproxcinod and NCX 429, but not naproxen, were able to induce relaxation of pre-contracted rabbit aortas (EC_{50}=5.5±1.7 and 6.2±1.6 μM, respectively), similarly to isosorbide mononitrate (EC_{50}=10.8±2.4 μM). The vasorelaxation was mediated by activation of the NO-signaling pathway, as prevented by the sGC inhibitor ODQ.
Interestingly, in activated RAW 264.7 macrophages, naproxcinod and NCX 429 (IC_{50}=0.8μM) but not naproxen inhibited LPS/IFNγ-mediated increase in nitrite concentrations. Consistently, naproxcinod reduced iNOS protein expression, whereas naproxen was ineffective.
Conclusions: The CINODs naproxcinod and NCX 429 inhibit expression of iNOS in activated macrophages *in vitro*, likely through a mechanism involving donation of NO. Therefore, in addition to the reported differentiated blood pressure and gastrointestinal profile over available NSAIDs, NO release from CINODs might confer additional anti-inflammatory properties.

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KNEE OSTEOARTHRITIS: SAFETY AND EFFICACY OF CLODRONATE IV

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Purpose: To assess the clinical effects of intravenous courses of clodronate in established knee osteoarthritis (OA) resistant to common treatment with non-steroidal anti-inflammatory drugs or local corticosteroids
Methods: Subjects aged 40 to 80 years with knee OA, from moderate to severe, were enrolled. The patients were treated with a 10 days course of clodronate i.v. 300mg/die every 3-6 months for 1 year. Patients were followed-up every 3 months. At each visit, pain scores (100 mm visual analogue scale [VAS]), Lequesne index scores, NSAID intake, physician and patient global assessments scores were recorded. Adverse events (AEs) were recorded throughout the study.
Results: 122 patients (females 107, males 15; mean age 74.3±5.1, Kellgren-Lawrence grade II or III OA) were enrolled. Statistically significant reductions in VAS pain scores, Lequesne index scores and NSAID usage were reported at all time-points (baseline VAS 67.02±10.4, 1 month VAS 46.5±15.3, 6 months VAS 41.7±11.3, 12 months 37.2±4.3 $p<0.01$). No systemic, serious or severe side effects were observed.
Conclusions: This study supports the safety, tolerability and effectiveness of Clodronate in the treatment of symptomatic knee OA. Clodronate may also offer economic benefits due to a reduction in NSAID usage and the resultant reduction in management costs of NSAID related side-effects. This

Figure 1

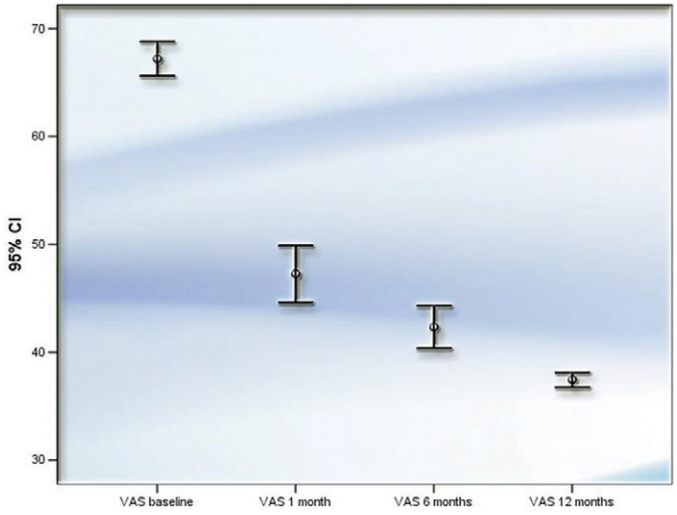

Figure 2

therapy could be also useful to protect cartilage damage as collagen type II.(1)

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OXIDATIVE STRESS INDUCED BY LOCAL ANESTHETICS IN OA CHONDROCYTES

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Purpose: Several different mechanisms have been proposed to explain toxicity of local anesthetics including the blockade of potassium channels and mitochondrial injury. The purpose of this study was to investigate whether oxidative stress is involved in toxic effects of lidocaine, bupivacaine, and ropivacaine on normal and OA chondrocytes.

Methods: Primary chondrocytes cultures, generated from cartilage from patients undergoing total knee replacement and normal donors, were exposed for 1 hr to saline, 2% and 1% of lidocaine, 0.5% and 0.25% of bupivacaine, 0.5% and 0.2% of ropivacaine. Enhanced mitochondrial superoxide production, oxidative damage to mitochondrial DNA and mitochondrial proteins were evaluated immediately after exposure or following 72 h of recovery. Mito SOX Red fluorescent dye, highly selective for mitochondrial superoxide, was used to study the mitochondrial ROS levels. Protein carbonylation as the result of oxidative stress was studied by Protein Carbonyl assay. Oxidative damage to mtDNA was evaluated using Quantitative Southern blot analysis.

Results: When normal and OA chondrocytes were exposed to local anesthetics, only small amount of mitochondrial ROS was accumulated in only OA chondrocytes immediately after exposure. Following 72 h after treatment, mitochondrial ROS production was enhanced. OA chondrocytes exhibited significantly higher levels of mitochondrial superoxide compare to normal donors. Enhanced mitochondrial ROS production in OA chondrocytes dose-dependently correlated with oxidative damage to mitochondrial DNA and proteins.

Conclusions: The present results demonstrate for the first time that mitochondrial oxidative stress is involved in toxic effects of local anesthetics on human chondrocytes. Moreover, OA chondrocytes exhibit higher susceptibility to this oxidative stress then chondrocytes obtained from normal donors. These differences are likely due to already compromised mitochondrial function and mitochondrial damage in OA chondrocytes.